

## THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

833 THIRD AVENUE  
NEW YORK, N. Y. 10017

## COMMITTEE:

Dr. Cattel, Chm.

Dr. Little

Dr. Bing

Application For Research Grant

Date: 27 April, 1967

1. Name of Investigator(s): (include Title and Degree) Irving Gellen, Ph.D.  
Chairman, Department of Pharmacology and Toxicology

## 2. Institution &amp; Address:

Southwest Foundation for Research and Education  
P.O. Box 2296 (10000 West Commerce Street)  
San Antonio, Texas 78206

3. Short Title of Project: Effects of Nicotine on Learning in Laboratory Animals

4. Proposed Starting Date: 1 September 1967

5. Anticipated Duration of this Specific Study: Three Years

6. Brief Description of Objectives or Specific Aims: The basis for this research proposal stems from the notion that nicotine may exert a predominantly tranquilizing effect in man and animals (1). To test such a hypothesis, one must of necessity employ an experimental paradigm of problem solving, learning, or some situation where the "tranquilized" or less anxious organism can perform at maximum capacity and ability. The experiments to be described in this proposal will illustrate the learning of a visual discrimination in hungry laboratory animals and some preliminary data which suggest that nicotine-injected animals may learn the task better than control animals. Such results might reflect a lowering of anxiety associated with the learning task and hence more rapid acquisition of the discrimination.

An extensive review of the literature reveals a paucity of research dealing with the effects of nicotine on learning or stress situations in animals (2,3,4,5,6). A comprehensive review of the existing studies has been published (7). A series of reports presented at a closed meeting in Colorado Springs (8) included a few investigations which dealt with the effects of nicotine on performance in laboratory animals. In such studies, subtle actions of nicotine tend to be overlooked and what usually results is a demonstration of the stimulant properties of nicotine. Investigators involved in such studies usually compare nicotine with known stimulants for effects on the performance of standard behavioral repertoires by animals.

A type of discrimination behavior is exemplified in a previous publication by this investigator and colleagues (9) who reported a delayed effect on behavior produced by small injections of nicotine bitartrate. In this experiment, hungry rats were trained to time precisely by rewarding with food those lever responses spaced 20 to 22 seconds apart. Injections of nicotine disrupted the timing behavior slightly, but pronounced delayed effects occurred three days after the drug injection and following a temporary return to base-line performance.

(Continuation on attached sheet)

7. Give a brief statement of your Working Hypothesis: "A consensus among modern authors appears to be that smoking and presumably nicotine exert a predominantly tranquilizing and relaxing effect(1)". The intent of this research is to test this hypothesis in a learning situation with laboratory animals. More rapid acquisition of the assigned task.

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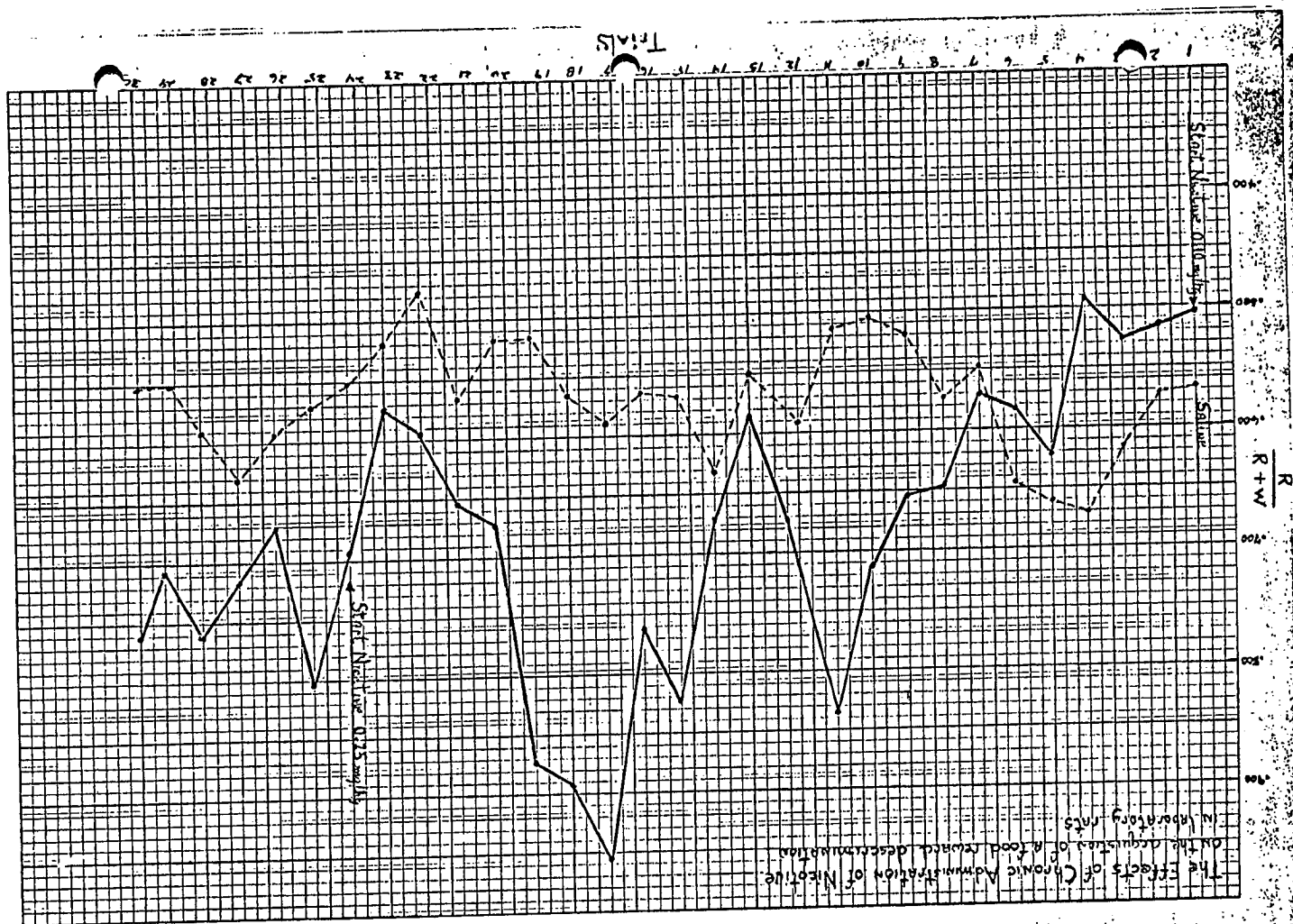
## Objectives and Specific Aims (Continued)

It was conjectured that these delayed changes in behavior following nicotine injections could conceivably be related to epinephrine output. Silvette et al., in their extensive review (1), found it "difficult to ascribe the above reported effects to any pharmacological action of a single injection of nicotine three days previously. A more likely conclusion would seem to be that the animals were effected by some other part of the experimental procedure rather than the nicotine per se."

It is of interest to note that a later study (10) reported an increase in excretion of urinary epinephrine produced by chronic injections of nicotine. The excretion reached a peak after three days of treatment.

The intent of the proposed research is to measure rate of acquisition of discrimination behavior in laboratory animals who are administered nicotine chronically during acquisition training.

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8. Details of Experimental Design and Procedures: (Attach Separate Pages) The subjects will be white rats, squirrel monkeys and cynomolgous monkeys. They will be gradually reduced to 80% of their body weight and maintained at this weight by limited feedings throughout the course of the experiment. The apparatus will consist of two operant conditioning chambers for rats, conditioning chambers for squirrel monkeys and two Foringer monkey chairs.

Two experimental procedures will be employed. Procedure A will involve the effects of chronic administration of nicotine on the learning of a visual or auditory discrimination based on positive reinforcement (food reward). Procedure B will involve the effects of chronic administration of nicotine on the learning of an auditory discrimination based on punishment ("conflict").

Procedure A. Hungry animals will learn to press a lever in order to obtain a liquid or solid food reward. Once the animals learn that depressing the lever is associated with the delivery of food rewards, the experimental conditions will be as follows: random presentations of light stimuli in the experimental chambers will serve as a signal that a lever response will be incorrect and will be punished by a 30 second period of darkness for the animals. If however, a tone stimulus accompanies the presentation of the light stimulus, a lever response will be correct and the hungry animal will be reinforced with food. For some animals light alone will indicate an incorrect response, while light and tone will indicate a correct response. For another group of animals tone alone will indicate an incorrect response, while tone plus light will indicate a correct response. (See Attachment)

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

The general facilities of the Southwest Foundation for Research and Education are available for this project. These include adequate office, laboratory and animal room space.

10. Additional Requirements:

None

11. Biographical sketches of all principal and professional personnel (append)

Attached

12. List of publications: (Five most recent as pertinent) (append)

Attached

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## 8. Experimental Design and Procedures (Continued)

All experimental animals will receive chronic administrations of nicotine during acquisition training, while control animals will receive saline.

Treatment of Data: In Figure 1 are preliminary data for two of four experimental rats which illustrate nicotine effects on the acquisition of such a discrimination. On the ordinate is shown efficiency or how well the animal is performing. These points are determined by taking the ratio of right responses divided by right and wrong or  $\frac{R}{R+W}$  = efficiency. If the animal is making an equal number of right and wrong responses, he is not discriminating and the point would fall at 0.50. If the animal's wrong responses exceed the right responses, the point would fall below 0.50. Points lying above .50 indicate that right responses exceed wrong responses and that the animal is acquiring the discrimination. The solid red line in the figure representing data for the nicotine rat shows that this animal acquired the discrimination faster than the saline animal. When the performance of the discrimination began to drop on the 20 to 23 day, the nicotine dose was increased from 0.1 to .25 mg/kg and the performance of the animal again became superior to that of the saline rat.

If, in the proposed experiment, the nicotine animals do acquire the discrimination more rapidly than the saline controls, it may be of interest to determine what effect the discontinuance of nicotine injections will have on the performance of the discrimination.

### Possible Results:

1. Nicotine treated animals will acquire the discrimination more rapidly than saline controls.
2. Saline controls will acquire the behavior more rapidly than nicotine animals.
3. There will be no significant differences between nicotine and saline animals in the rate of acquisition of the discrimination.

Procedure B: This procedure, developed by this investigator and colleagues in 1957, is currently used extensively in the pharmaceutical industry for the pre-clinical evaluation of the minor tranquilizers (anti-anxiety agents). The behavior involves the learning of an auditory discrimination (or visual) based on punishment. The auditory discrimination is conditioned in hungry laboratory animals by simultaneously rewarding with food and punishing with mild electric shock all lever responses made in the presence of a discriminative stimulus (tone or light). Appropriate setting of the shock intensity results in suppression of ongoing lever pressing in the presence of the discriminative stimulus. The intent of this experiment is to investigate the rate of acquisition of such a discrimination in animals administered nicotine chronically.

The hungry laboratory animals first learn to press a lever in order to obtain food rewards which are obtainable on the average of once every two minutes (2 minute VI). When lever pressing rates have stabilized a tone or light stimulus of 3 minute duration is introduced at regular intervals during the lever-pressing session. This stimulus serves as a signal for the animal that all lever responses will be reinforced with food. The discriminative stimulus signals a change from a relatively undesirable schedule of reinforcement (2 minute VI) to a schedule with a higher "pay-off" of reinforcements (continuous reinforcement). When the behavior has stabilized, a

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## 8. Experimental Design and Procedures (Continued)

punishment contingency is added so that during the discriminative stimulus, lever pressing is rewarded with food but also produces mild shock to the feet of the animal. The hungry animal must balance the positive aspects of obtaining food against the negative aspects of accepting electric shocks in order to obtain the food. After a period of time these experimental contingencies result in a suppression of lever-pressing behavior during stimulus periods. Anti-anxiety agents will reinstate lever pressing behavior that has been suppressed by punishment (11,12,13).

This investigation will test the effects of nicotine on the acquisition of such a discrimination in laboratory animals. Animals will be administered nicotine chronically during acquisition training and will later be compared with saline controls.

### Possible Results:

1. Nicotine animals will acquire the punishment discrimination more rapidly than saline controls.
2. Saline animals will acquire the punishment discrimination more rapidly than nicotine animals.
3. There will be no significant difference between saline and nicotine animals in acquisition of the punishment discrimination.

The data from these experiments will show whether nicotine treated animals will acquire a discrimination more rapidly than saline controls. They will also show if the findings are similar for discriminations based on positive reward as well as punishment. They will yield information with regard to reproducibility of the phenomenon between species. Findings may or may not be qualitatively similar for rats, squirrel monkeys, and old world monkeys.

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## REFERENCES

1. Silvette, H., Larson, P. S. and Haag, H. B.: Medical uses of tobacco past and present. *Vir. Med. Monthly*, 85, 472-484, 1958.
2. Smith, C. S., Rosenfeld, S. and Sacks, L. J.: Study of the effect of nicotinism in the albino rat. *J. Pharmacol.* 55, 274-287, 1935.
3. Kuschinsky, G. and Hotovy, R.: Uber die zentral erregende Wirkung des nicotins. *Klin Wschr.* 22, 649-650, 1943.
4. Heimstra, H. W., Grodsky, M. A. and Davis, R. T.: Sand digging behavior of rats. *Proc. S. Dak. Acad. Sci.* 34, 96-102, 1955.
5. Cervalo, B. A. and Cheskey, C.: Effects of nicotine sulfate injections on sand digging behavior of rats. *Proc. S. Dak. Acad. Sci.* 36, 222-225, 1957.
6. Bonta, I. L., Delver, A., Simons, L. and De Vos, C. J.: A newly developed motility apparatus and its applicability in two pharmacological designs. *Arch. Int. Pharmacodyn.* 129, 381-394, 1960.
7. Silvette, H., Hoff, E. C., Larson, P. S. and Haag, H. B.: The actions of nicotine on central nervous system functions, *Pharm. Rev.* 14, 137-173, 1962.
8. Research Conference and Workshop on Nicotine. Sponsored by AMA and held in Colorado Springs, November 1-3, 1966.
9. Geller, I., De Marco, A. O. and Seifter, J.: Delayed effects of nicotine on timing behavior in the rat. *Science*. 131: 1960
10. Westfall, T. C. and Watts, D. T.: The effect of nicotine on amines of brain and urine in the rat. *J. of Neurochem.* 11, 397-402, 1964.
11. Geller, I. and Seifter, J.: Effects of meprobamate, barbiturates, d-amphetamine and promazine on experimentally induced conflict in the rat. *Psychopharmacologia*. 1, 1960.
12. Geller, I. and Seifter, J.: The effects of monourethanes, diurethanes and barbiturates on a punishment discrimination. *J. Pharmacol. Exptl. Therapeutics*. 136, No. 3, 1962.
13. Geller, I.: Use of approach avoidance behavior (conflict) for evaluating depressant drugs. Chapter 33, in Nodine (ed.), *Psychosomatic Medicine*, The First Hahnemann Symposium, Lea and Febiger: Philadelphia, 1962.

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## CURRICULUM VITAE

Irving Geller, Ph. D.

Date of Birth: R Place: Boston, Massachusetts.

<u>Education:</u>	<u>Institution</u>	<u>Degree</u>	<u>Date</u>	<u>Major Field</u>
	George Washington Univ.	B. A.	1949	Pre-med.
	George Washington Univ.	M. A.	1951	Psychology
	American University	Ph. D.	1957	Psychology

Present Position (s):

Chairman of the Department of Pharmacology, Division of Biological Growth and Development, Southwest Foundation for Research and Education. August 1966 - .

Previous Positions:

Aviation Psychologist - Naval Res. Lab., Washington, D. C.; Forbes Air Force Base, Topeka, Kansas, March 1951-November 1951.

Research Psychologist - Walter Reed Army Inst. of Research, Washington, D. C. 1952-1957.

Senior Research Scientist - Wyeth Labs. Inc., Radnor, Penna. 1957-1964.

Associate Research Professor of Pharmacology - New York Medical College, 1964 - 1966.

Professional Societies:

American Psychological Association

New York Academy of Science

Psychonomic Society

Sigma Xi

American Society for Pharmacology and Experimental Therapeutics.

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## PUBLICATIONS

Irving Geller, Ph. D.

1. Geller, Irving, M. Sidman and J. V. Brady, "The Effects of Electroconvulsive Shock on a Conditioned Emotional Response: A Control for Acquisition Recency," *J. Comparative and Physiol. Psychol.*, Vol. 48, No. 2, 130-131 (1955).
2. Geller, Irving, and J. Seifter, "The Effects of Promazine and Phenergan on Multiple Schedule Reinforcement Performance in the Albino Rat," *Fed. Proc.*, Vol. 18, (1959).
3. Geller, Irving, A. O. DeMarco and J. Seifter, "Delayed Effects of Nicotine on Timing Behavior in the Rat," *Science*, Vol. 131, No. 3402, 735-737 (1960).
4. Geller, Irving, and Joseph Seifter, "The Effects of Meprobamate, Barbiturates, d-Amphetamine and Promazine on Experimentally Induced Conflict in the Rat," *Psychopharmacologia*, 1, 482-492, (1960).
5. Geller, Irving, "The Acquisition and Extinction of Conditioned Suppression as a Function of the Base-Line Reinforcer," *J. Exptl. Anal. Behavior*, Vol. 3, No. 3, 235-240 (1960).
6. Geller, Irving, "Behavioral Procedures Used in Evaluation of the Psychopharmacological Effects of Carphenazine," *Diseases of the Nervous System, Supplement*, Vol. XXII, No. 2 (1961).
7. Geller, Irving, and J. V. Brady, "Effect of Electroconvulsive Shock on an Extinguished "Fear" Response," *Science*, Vol. 133, No. 3458, 1080-1081 (1961).
8. Geller, Irving, "Use of Approach Avoidance Behavior (Conflict) for Evaluating Depressant Drugs," *1st Hahnemann Symp. on Psychosomatic Medicine*, Chap. 33, 267-274 (1962).
9. Geller, Irving, J. T. Kulak, Jr. and J. Seifter, "The Effects of Chlordiazepoxide and Chlorpromazine on a Punishment Discrimination," *Psychopharmacologia*, 3, 374-385, (1962).
10. Geller, Irving, "Experimentally Induced Conflict for Evaluation of Psychopharmacologic Agents" (A Scientific Exhibit) (1962).
11. Geller, Irving, E. Bachman and J. Seifter, "Effects of Reserpine and Morphine on Behavior Suppressed by Punishment," *Life Sciences*, No. 4, 226-231 (1963).
12. Geller, Irving and J. Seifter, "The Effects of Mono-Urethans, Di-Urethans and Barbiturates on a Punishment Discrimination," *J. Pharm. and Exptl. Therapeutics*, Vol. 136, No. 3, 284-288 (1962) (one copy only).
13. Geller, Irving, "Conditioned "Anxiety" and Punishment Effects on Operant Behavior of Goldfish (*Carassius auratus*)," *Science*, 141, 3578, 351-353 (1963).

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14. DeMarco, A. O. and I. Geller, "Effects of Acceleration Forces on Timing Behavior in the White Rat," Aerospace Medicine, Vol. 35, No. 1, 30-32 (1964).
15. Geller, Irving, "Relative Potencies of Benzodiazepines as Measured by Their Effects on Conflict Behavior," Arch. int. Pharmacodyn, 149, No. 1-2, 243-247 (1964).
16. Geller, Irving, "Conditioned Suppression in Goldfish as a Function of Shock-Reinforcement Schedule," J. Experimental Analysis of Behavior, Vol. 7, No. 5, 345-349 (1964).
17. Geller, Irving, "Psychopharmacology of Tybamate," Journal of Psychopharmacology, Vol. 1, No. 2, 47-55 (1966).

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## 13. Budget (1st year)

## A. Salaries (Personnel by names)

## Professional

Irving Geller, Ph. D.

% time

25%

Amount

(Salaries include fringe benefits)

## Technical

Research Assistance

100%

Animal caretaker and Laboratory Helper

100%

Sub-Total

## B. Consumable Supplies (list by categories)

Animals - Rats and Monkeys

1. Animal food - wood shavings -  
syringes - vitamins - food reinforcers.

2500.00

2. Recorder paper, ink, pens, electrical supplies and  
replacement parts

500.00

Sub-Total

3000.00

## C. Other Expenses (itemize)

Travel - visit to scientific meetings

500.00

Publication costs - illustrations, slides, etc.

500.00

Sub-Total

1000.00

## D. Permanent Equipment (itemize)

Cages for housing squirrel monkeys and rats -  
includes devices for watering and feeding.

4500.00

Operant conditioning chambers for rats

1500.00

Operant conditioning chambers for squirrel monkeys (we have)

00.00

Behavioral Control equipment and recorders (see attachment)

8000.00

14000.00

2014.00

## E. Overhead (15% of A + B + C).

Total

\$29,442.00

## Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	\$2000.00	\$1200.00	\$1500.00	\$2438.00	\$20,189.00
Year 3	R	\$2300.00	\$1000.00	\$1000.00	\$2535.00	\$20,435.00

It is understood that the applicant and institutional officers  
in applying for a grant have read and found acceptable  
the Council's "Statement of Policy Containing Conditions  
and Terms Under Which Project Grants Are Made."

Signature

Director of Project

Signature

Business Officer of the Institution  
EDWARD F. FEITH  
TREASURER

Telephone

Telephone

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Behavioral Control Equipment and Recorders

<u>Qty</u>	<u>Description</u>	<u>Unit Price</u>	<u>Total Price</u>
3	Cumulative Recorders	\$ 450.00	\$ 1350.00
3	4027 JM Reg. Power Supply	203.50	610.50
3	4005 J Timer	156.25	468.75
3	4005 JM Timer	193.75	581.25
6	4013 J Pulse Generator	51.25	307.50
3	4069 J Dual Pulse Generators	60.00	180.00
6	4001 J and Gate	43.75	262.50
6	4035 J Dual and Gate	43.75	262.50
1	4043 J Dual Exc. OR Gate	44.45	44.45
6	4011 J Diode Panels	30.00	180.00
6	4024 J Flip Flop R/S Binary	56.25	337.50
4	4072 J Basic Timer	101.25	405.00
3	4020 J Probability Generator	124.85	374.55
4	4023 J Delay Generator	58.75	235.00
6	4018 J Cradle Relay	31.00	186.00
6	4066 J Binary Relay (Alt.)	65.00	390.00
6	4068 J Session Timers	64.50	387.00
3	4028 J Pre Deter Counters	248.00	744.00
11	4010 J Reed Logic Relays	25.00	275.00
3	4025 J Noise Generator	139.50	418.50

GRAND TOTAL

\$ 8000.00

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# Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Experimental Studies in Psychopharmacology	NIH	\$32,950 September, 1966 to August, 1967	Terminates August 31, 1967. Renewal approved but funds to be reduced more than 50%.
Effects of Nicotine on Timing Behavior in Rats	AMA	\$25,000	May, 1966 - April, 1968

Pending

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